PROTEIN AND NONPROTEIN NITROGEN CONTENTS OF SOME OILSEEDS AND PEAS

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Nonprotein nitrogen (NPN) was extracted from seven species of oilseeds and three cultivars of peas (*Pisum sativum* L.) by three methods. Method 1 was extraction of meal nitrogen with dilute sodium hydroxide and removal of alkali-soluble proteins by trichloroacetic acid (TCA) precipitation. Methods 2 and 3 were extractions of meal nitrogen with 70% ethanol and 1% TCA, respectively. The three solvents extracted vastly different quantities of nitrogen from the meals. Method 3 gave the highest values for NPN followed by methods 1 and 2 in that order. The nitrogen extracted by ethanol was probably the true NPN content of the meals because of the lack of solubility of oilseed and pea proteins in this solvent. The oilseed meals contained more amide nitrogen than the peas. None of the meals contained any significant quantities of nitrate nitrogen. Amino acid analysis of NPN fractions of meals obtained by method 1 showed the oilseed meals and peas to contain, in free state, all the protein amino acids except cystine or an appreciable amount of methionine. The NPN fractions of the meals contained, except in safflower (*Carthamus tinctorius* L.), high quantities of ammonia, glutamic, and aspartic acids. Safflower NPN fraction contained, in addition to ammonia, more proline and alanine than glutamic and aspartic acids. Mustard (*Brassica juncea* Coss.) and pea NPN fractions also contained high concentrations of arginine. The other protein amino acids were present in trace or relatively small concentrations. The major conclusion drawn from the data was that the NPN of the seed species used in the study was highly variable and depended on the method and solvent of extraction.

Les auteurs ont extrait, suivant trois méthodes, l'azote non protéique de sept espèces d'oléagineux et de trois cultivars de pois (*Pisum sativum* L.). La première méthode consistait en une extraction de l'azote des tourteaux au moyen d'une solution diluée d'hydroxyde de sodium et la précipitation des protéines solubles en milieu alcalin au moyen de l'acide trichloroacétique (TCA). Les méthodes 2 et 3 consistaient en des extractions de l'azote à l'éthanol 70% et à l'acide trichloroacétique 1%, respectivement. Les trois solvants ont extrait des quantités très différentes d'azote. La troisième méthode a donné les valeurs d'azote non protéique les plus élevées, et a été suivie dans l'ordre des méthodes 1 et 2. L'azote extrait à l'éthanol représentait probablement la teneur réelle en azote non protéique des tourteaux, à cause du manque de solubilité des protéines des oléagineux et des pois dans ce solvant. Les tourteaux d'oléagineux contenaient plus d'azote d'amide que les pois. La teneur en azote nitrique n'était significative dans aucun des tourteaux. L'analyse des acides aminés des fractions d'azote non protéique obtenues par la première méthode a montré que les tourteaux d'oléagineux et les pois contenaient tous les amino acides protéiques à l'état libre, à l'exception de la lysine, ou une quantité appréciable de méthionine. Les fractions d'azote non protéique des tourteaux contenaient, sauf dans le cas du carthane (*Carthamus tinctorius* L.), des quantités élevées d'ammoniac, d'acide glutamique, et d'acide aspartique. La fraction d'azote non protéique du carthame contenait, en plus de l'ammoniac, plus de proline et d'alanine que d'acides glutamique et aspartique. Les fractions d'azote non protéique de la moutarde (*Brassica juncea* Coss.) et des pois avaient aussi des concentrations élevées en arginine. Les autres

amino acides protéiques étaient présents à l'état de traces ou en concentrations relativement faibles. La principale conclusion à tirer de ces données est que la teneur en azote non protéique des espèces étudiées variait considérablement et était fonction de la méthode et du solvant employés.

INTRODUCTION

Oilseeds and legumes are a rich source of proteins and essential amino acids (Van Etten et al. 1967). Some of these species are low in certain essential amino acids, for instance, peas (*Pisum sativum* L.) and soybean (*Glycine max* Merr.) in methionine and cystine (Van Etten et al. 1967; Wolf 1970), and sunflower (*Helianthus annuus* L.) and safflower (*Carthamus tinctorius* L.) in lysine and methionine (Evans and Bandemer 1967; Tkachuk and Irvine 1969; Sosulski and Sarwar 1973). Nevertheless, they provide, in combination with each other and cereals, a nutritious product for livestock feed as well as for human foods. An increasing use of oilseed proteins and their products is forecast to partly replace the costly animal proteins in human diets on the North American continent and to raise total protein intake of people in the developing countries (Dimler 1971).

One method of using seed proteins is to prepare protein-rich concentrates and isolates from the seeds for incorporation into a wide variety of food products as has been done with soybean (Wolf 1970). These products are prepared by extracting nitrogenous materials from ground seed or meal with a dilute alkaline solvent of appropriate *p*H followed by isoelectric precipitation of the soluble proteins by addition of an acid (Anson 1962). The precipitated proteins are then removed by centrifugation. This process leaves in the supernatant a nonprecipitable, nonprotein nitrogen (NPN) fraction containing peptides, free amino acids, and many other nitrogen-containing compounds. Sosulski and Bakal (1969) reported substantial quantities of nitrogen lost in the NPN (or whey) fractions obtained on preparation of isolates from a number of oilseeds. For instance, the NPN formed 10–12% of the total meal nitrogen in soy, 23–32% in rape (*Brassica napus* L. and *Brassica campestris* L.) and flax (*Linum usitatissimum* L.), and 17–20% in sunflower. More recently, Nakai et al. (1972) reported loss of proteins, methionine, and cystine in the whey fraction on preparation of isolates from rape flour. Because of the high cost of purification and concentration the NPN fraction cannot be recovered economically and therefore constitutes a serious loss of meal protein.

The objective of the present study was to determine the type and relative concentration of amino acids present in NPN fractions prepared from seven species of oilseeds and three cultivars of peas. The method employed was similar to that commonly used for the preparation of protein isolates, except that the proteins were removed with TCA rather than by isoelectric precipitation with HCl. The NPN was also directly extracted from the meals with ethanol and TCA, and yields compared with those obtained by alkaline extraction of the meal nitrogen followed by removal of the proteins with TCA. In addition, information was obtained on the nitrate nitrogen and amide nitrogen contents of the meals.

MATERIALS AND METHODS

The rapeseed cultivar Zephyr, turnip rapeseed cultivar Span, Oriental mustard (*Brassica juncea* Coss.), Noralta flax, commercial sunflower, safflower, soybean, and three cultivars of peas, Century (yellow smooth), PI 106801 (green wrinkled), and PI 165949 (speckled) were obtained from the University of Saskatchewan, Saskatoon, increase plots. All the species and cultivars were grown under comparable conditions. The pea cultivars were originally obtained from the United States Department of Agriculture World Collection.

Sunflower and safflower were mechanically dehulled and the hulls were removed by aspiration. All seeds were finely ground to a meal in a coffee mill (Krups model 318). Oilseed meals were defatted by homogenizing twice with excess of skelly-solve F (bp 30–60 C) in a Waring blender. The solvent was removed by vacuum filtration and the meals were dried at room temperature.

Total nitrogen content of the meals was determined by a micro-Kjeldahl method (Association of Official Agricultural Chemists 1970). Nitrate nitrogen was determined after treatment of the meals with the salicylic–sulfuric acid mixture described by Humphries (1956). Amide nitrogen was determined by refluxing the meals (50 mg) with 2 N HCl for 90 min (3 ml/mg protein). The digest was filtered through glass wool, made to *p*H 9–11 with 2 N sodium hydroxide, and the ammonia distilled into boric acid solution in a micro-Kjeldahl distillation unit.

NPN was determined by three methods.

Method 1 (NaOH–TCA) — Three g of each meal was extracted with 75–80 ml of 0.2% (w/v) sodium hydroxide (*p*H 12.0) for 90 min in a wrist-arm shaker. The extract was centrifuged at 10,000 × *g* for 20 min to remove the insoluble materials. An aliquot of the supernatant fraction was analyzed for total nitrogen (alkali-soluble nitrogen). To the remaining supernatant an equal volume of freshly prepared 30% (w/v) TCA was added and the mixture was stirred at room temperature for 5 min for a thorough precipitation of the proteins. The proteins precipitated were removed by centrifugation (10,000 × *g*; 10 min). An aliquot of the supernatant was analyzed for total nitrogen to obtain nitrogen not precipitated by the TCA (NPN).

Method 2 (EtOH) — NPN was directly extracted from the meal with 70% (v/v) ethanol at a meal to solvent ratio of 1:40 by shaking in a wrist-arm shaker for 60 min. The insoluble materials were removed by centrifugation (10,000 × *g*; 10 min). An aliquot of the supernatant was analyzed for total nitrogen.

Method 3 (TCA) — This method was the same as method 2 except that the solvent was 1% (w/v) TCA.

For amino acid analysis the supernatant fraction obtained in method 1 was repeatedly extracted in a separatory funnel with water-saturated ether to remove the TCA. The aqueous layer containing the NPN was collected, reduced in volume on a rotary evaporator, and an aliquot analyzed for free amino acid content on a Beckman Model 120 C amino acid analyzer.

RESULTS AND DISCUSSION

Expressed on an as is basis, the protein content of the oilseed meals varied from 33 to 51%, and those of the peas from 22 to 25% (Table 1). The rape meals contained 3–4% residual oil and on oil-free basis, turnip rape contained 5% less protein than rape. Sunflower and safflower meals contained the highest protein content, followed by soy, mustard, flax, rape, and peas in that order. Century peas contained less protein than the two other cultivars of peas. None of the meals contained any significant quantities of nitrate nitrogen, which was detected only in rape and mustard. All the species examined contained amide nitrogen. This nitrogen fraction was partly responsible for the high ammonia content of the NPN fractions. The oilseed meals contained higher concentrations of amide nitrogen than the peas (Table 1).

Sodium hydroxide solubilized, except in soy and mustard, 77–87% of the meal nitrogen in oilseed meals and peas. The alkaline

Table 1. Nitrogen fractions of oilseed meals and peas; data are averages of duplicate determinations

Species & cult	mg/g meal			Alkali-soluble nitrogen, %	Nonprotein nitrogen (% of meal nitrogen)		
	Meal protein	Nitrate-nitrogen	Amide-nitrogen		NAOH–TCA	EtOH	TCA
Rapeseed	379	0.6	6.0	76.9	6.4	4.7	29.6
Turnip rapeseed	329	0.0	4.4	72.5	6.6	5.1	32.9
Mustard	409	0.5	5.8	68.7	6.6	4.8	29.5
Flax	364	0.0	5.1	77.3	7.1	1.4	15.6
Sunflower	513	0.0	7.2	82.3	5.5	2.6	16.4
Safflower	520	0.0	6.9	87.4	6.1	2.9	9.8
Soybean	449	0.0	5.8	67.0	6.8	1.9	12.8
Peas							
Century	226	0.0	2.5	83.8	13.2	3.8	19.5
PI 106801	274	0.0	2.6	82.2	10.2	3.7	15.2
PI 165949	253	0.0	2.5	75.4	10.2	3.2	12.5

solubility of soy nitrogen obtained in the present study was rather low; 80–90% of soy nitrogen can be solubilized after repeated extractions, either with dilute hydrochloric acid, *p*H 2–3, or with sodium hydroxide, *p*H 8–12 (Wolf 1970). Alkaline solubility of mustard meal nitrogen has not been reported in the literature.

The NPN extracted from the meals according to method 1 varied between 5 and 7% of the meal nitrogen in oilseed meals, and between 10 and 13% of the meal nitrogen in the peas (Table 1). There were some differences in the NPN content among Century and the other two cultivars of peas, but the NPN content of the two cultivars of rapeseed was essentially the same. In general, all species of oilseeds contained essentially similar quantities of NPN, and peas, on the average, contained almost twice the quantities of NPN than the oilseeds. Extraction of the meals with 70% ethanol (method 2), a solvent commonly used for the extraction of NPN from plants, gave about 5% NPN in rapeseed meals and mustard, less than 3% in other oilseed meals, and 3–4% in peas. Unlike in method 1, there were species differences in the NPN content of the meals extracted with ethanol. For example, soy and flax meals contained less alcohol-soluble nitrogen than sunflower and safflower. These latter oilseed meals contained less alcohol-soluble nitrogen than rapeseed meals and mustard. The three cultivars of peas contained an essentially similar content of NPN, although it was higher than the NPN content of flax, sunflower, safflower, and soybean but lower than the NPN content of rape and mustard. When the meals were directly extracted with 1% TCA (method 3), the nitrogen solubilized was much higher than in methods 1 and 2. TCA solubilized almost 30% of the meal nitrogen in rape and mustard, 10–15% in other oilseed meals, and 12–20% in peas. There were substantial differences in the TCA-soluble nitrogen content of the oilseed species, particularly rape and mustard, and the three cultivars of peas. Interestingly, the TCA-soluble nitrogen obtained in the present study closely compared, except in flax, with the nitrogen content of whey obtained from some of these oilseeds during protein isolation (Sosulski and Bakal 1969). An earlier study (Bhatty and Finlayson 1973) reported that low concentrations of TCA, such as that used in the present study, extract large quantities of protein nitrogen from rape, sunflower, and soy meals. It is therefore likely that the high nitrogen content of TCA extracts of meals was due to substantial extraction of protein nitrogen from the meals by this solvent. Concentrations of TCA at which protein solubility is minimal (13%) have been employed by Becker et al. (1940) for the extraction of NPN from soy meal. However, such concentrations of TCA are difficult to remove, especially by extraction with ether if the NPN fraction is to be analyzed for free amino acid content or used for further chemical analysis. Excessive washings with ether are required and this may cause loss of nitrogen, especially amino nitrogen, because of the presence of some ethanol as an impurity in diethyl ether. In the present study, to remove TCA from the NPN fractions obtained in method 1, 10–12 washings with ether were required in each instance to raise the *p*H of TCA supernatant from 1.2 to about 6.0. The higher NPN values obtained in method 1 compared with those obtained with ethanol extraction (method 2), particularly in peas, may be due to the lack of precipitation by TCA of small-molecular-weight peptide materials present in these extracts. Some of these peptide materials probably arose from partial hydrolysis of proteins by the alkaline solvent during extraction of the meals. Alternatively, lower values of NPN obtained with ethanol extraction may be partly due to lower solubility of basic amino groups such as lysine and arginine in ethanol. An earlier study (Bhatty and Finlayson 1973) showed that ethanol and some other organic solvents extract lower quantities of basic amino acids from oilseed meals than TCA. Nevertheless, the nitrogen extracted by ethanol probably represents the true NPN content of the meals because of the lack of solubility of oilseed and pea reserve protein in ethanol. The other two methods extracted, in addition to NPN, large quantities of protein nitrogen.

Table 2. Free amino acid composition of the nonprotein nitrogen fractions of oilseed meals and peas; data are averages of duplicate determinations

Amino acids	Rapeseed	Turnip rapeseed	Mustard	Flax	Sunflower	Safflower	Soybean	Peas		
								Century	PI 106801	PI 165949
					μmoles per g meal					
Tryptophan	0.15	0.13	0.57	0.31	1.30	0.31	0.98	0.44	0.13	0.23
Lysine	0.21	0.36	0.31	0.08	0.43	0.12	0.29	0.42	0.71	0.35
Histidine	0.05	0.11	0.14	0.11	0.23	0.34	0.14	0.09	0.17	0.08
Ammonia	6.76	9.99	12.76	5.16	14.21	12.04	9.13	5.75	8.09	9.55
Arginine	0.28	0.11	20.32	0.92	1.85	2.41	3.37	24.45	19.26	17.71
Aspartic acid	3.98	4.23	3.91	3.33	5.58	1.05	2.69	1.89	4.03	5.42
Threonine	–	–	–	–	–	–	–	–	–	–
Serine	20.03	15.69	23.67	2.05	5.96	1.01	3.82	13.70	14.33	11.05
Glutamic acid	0.32	Trace	0.60	0.87	0.56	10.33	0.34	Trace	0.38	0.29
Proline	0.27	0.22	0.40	0.97	1.37	0.77	0.44	0.86	1.73	1.34
Glycine	0.65	0.52	0.58	0.53	1.97	2.85	1.55	0.85	2.40	0.88
Alanine	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Half cystine	0.20	0.22	0.27	Trace	0.71	0.58	2.62	0.50	0.43	0.37
Valine	Trace	0.07	0.10	Trace	0.19	Trace	0.14	0.09	0.07	Trace
Methionine	Trace	0.10	0.14	Trace	0.55	0.28	0.16	0.16	0.16	0.16
Isoleucine	Trace	0.11	0.11	Trace	0.24	0.13	0.21	0.23	0.33	0.23
Leucine	Trace	0.14	0.13	Trace	0.32	0.16	Trace	0.15	0.40	0.16
Tyrosine	Trace	0.20	0.42	Trace	0.62	0.47	0.26	0.35	0.40	0.16
Phenylalanine	0.29	0.20	64.44	14.33	36.11	32.87	26.10	49.93	52.81	48.08
Total	33.20	32.21								

The NPN values obtained in method 1 suggest that TCA removes soluble protein more completely from alkaline extracts of meal than when proteins are removed by isoelectric precipitation by addition of hydrochloric acid. For this reason the NPN values obtained by method 1 were much lower than nitrogen content of whey obtained from some of these oilseeds by Sosulski and Bakal (1969). Incomplete removal of extracted protein by isoelectric precipitation undoubtedly contributes to a larger loss of seed nitrogen in the whey fraction, and consequently a lower recovery of nitrogen in the isolates. Alternate methods of protein removal from alkaline extracts should, therefore, be investigated in the preparation of protein isolates from these seeds. TCA may be used as a protein precipitant; it can be easily removed from the proteins by dialysis. Alternatively, proteins may be removed from the alkaline extracts by heating in the presence of calcium salts (Anson 1962).

The oilseed meals and peas contained in the NPN fraction all the protein amino acids except cystine and methionine and the latter was present in most instances in quantities of less than 0.5 μmole (Table 2). Rapeseed and flax contained only trace amounts of valine, isoleucine, leucine, tyrosine, and phenylalanine. Table 2 does not include values for threonine and serine because of the incomplete separation of these amino acids due to interference of unknown compounds, presumably peptides, eluted from the column. Although a number of unknown compounds were eluted from the column; usually before aspartic acid, none of the other protein amino acid peaks was distorted, as is shown by their resolution and retention times. The NPN fractions, particularly those of mustard, sunflower, and safflower, contained high concentrations of ammonia, which was probably derived mostly from the hydrolysis of amide nitrogen during extraction of the meals with sodium hydroxide. Some of these meals, particularly those of sunflower and safflower, contained high amide nitrogen (Table 1). The high ammonia content of other NPN fractions, especially those of pea cultivars PI 106801 and PI 165949, may also be due to the presence of some free ammonia in these meals.

The major amino acids of the meal extracts were glutamic and aspartic acids, except in safflower, which contained substantial quantities of proline and alanine. In addition to these two amino acids, mustard and all three cultivars of peas contained extremely high concentrations of arginine. For instance, mustard contained about 200 times more arginine than rape, 20 times more than flax, and 8–12 times more than sunflower, safflower, and soybean. The other amino acids were present in relatively small concentrations (less than 1 μmole). None of the meal NPN fractions contained cystine in the free state, or an appreciable amount of methionine, which is inconsistent with the results of Nakai et al. (1972).

The present results and those of the previous study (Bhatty and Finlayson 1973) show that the NPN content of oilseeds and peas and probably of other seeds is a variable fraction and depends on the method and solvent of extraction. Although the NPN fractions obtained from oilseeds and peas used in the study apparently contained many ninhydrin-positive materials, the major amino acids detected were glutamic and aspartic except in safflower and, in addition, arginine in mustard and peas.

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